Reviewer Report

Title: **Comprehensive evaluation of RNA-Seq analysis pipelines in diploid and polyploid species**

Version: **Original Submission** **Date:** 6/2/2018

Reviewer name: **Ruibang Luo**

Reviewer Comments to Author:

Major Concern:
The authors benchmarked Control Reads against Treatment Reads, Single Sample against Multiple Samples as input, CD-HIT against RapClust for clustering, and five mappers including bowtie2, gsnap, stampy, star and hisat2 for mapping reads. But for assembly, the authors benchmarked only one transcriptome assembler, Trinity. The authors claimed, "Trinity is commonly selected and has good performance" in line 94 and cited two papers. One paper titled "Optimizing de novo transcriptome assembly …" was published 2011, which is a bit outdated and doesn't include the benchmark of latest short-read transcriptome assemblers. The other paper "Comprehensive evaluation of de novo …" is new (2017) but doesn't support the authors claim and concluded in its abstract, quote: "SOAPdenovo-Trans performed best in base coverage, while Trans-ABySS performed best in gene coverage and number of recovered full-length transcripts. In terms of chimeric sequences, BinPacker and Oases-Velvet were the worst, while IDBA-tran, SOAPdenovo-Trans, Trans-ABySS and Trinity produced fewer chimeras across all single k-mer assemblies." As we know, transcriptome assemblers perform differently on genomes of different characteristics - Trinity usually performs better on mammals and vertebrates, SOAPdenovo-Trans on plants and Trans-ABySS on metagenomics. As the authors are targeting a "Comprehensive evaluation of RNA-Seq analysis pipelines", it is necessary to include another one or two leading transcriptome assemblers.

Minor Concerns:
Cite Detonate score paper in line 240.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

Conclusions

Are the conclusions adequately supported by the data shown? Choose an item.

Reporting Standards

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Statistics

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